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CHARACTERIZATION AND AN ASSESSMENT OF ANTI-INFLAMMATORY EFFECT OF AN OPTIMIZED THYMOL LOADED SODIUM ALGINATE-CHITOSAN COPOLYMERIC NANOFORMULATION

Jyoti Sheorain[a], Meenakshi Mehra[a] and Santosh Kumari[a]
[a]Department of Bio and Nano technology, Guru Jambeshwar University Of Science and Technology, Hisar, Haryana 125001
Email:jyotisheorain72198@gmail.com Mob:9992843200

Nanotechnology can be helpful in enhancing stability,solubility and dose reduction.This present investigation demonstrates that an optimized Sodium alginate-Chitosan nanoformulation possesses improved efficacy. Thymol is a well-recognized, researched and USFDA GRAS notated natural substance having important properties like microbicidal, negligible toxicity, anti-inflammatory, free radical scavenging etc. Sodium alginate is natural polysaccharide with multiple anionic or cationic groups and chitosan, a natural cationic polymer.Both aforementioned polymers are ideal carriers being low-cost,biodegradable,biocompatible and non-toxic. Ionic gelation method was used to synthesize thymol loaded sodium alginate- chitosan copolymeric nanoformulation. Optimized nanoformulation of 2:1 ratio (Sodium alginate:Chitosan) was characterized and in-vitro evaluated for anti-inflammatory effects. It has 89% entrapment efficiency, mean hydrodynamic diameter of 209nm, and zeta potential of 34.5mV. Further anti-inflammatory activity of optimized nanoformulation was found highly significant at 500 µg/ml concentration using HRBC membrane stabilization method.The present study concludes that an encapsulation of thymol in matrix of natural polymers(chitosan and sodium alginate) can play an important role in development of novel natural anti-inflammatory agent.

Keywords: Sodium alginate; Chitosan; Thymol; Copolymeric Nanoformulation; Anti-inflammatory.

Introduction

With the advent of nanotechnology, numerous futuristic biopolymers of the scale of nanometers are being developed in lieu of non-biodegradable polymers due to manifold benefits like lesser toxicity and better bioactivity, biodegradability, versatility, high elasticity, wide spectrum bio-compatibility, huge availability consequently lesser costs and sustainability [1-3]. They are also capable of encapsulating both hydrophilic and hydrophobic compounds with less probability of off-target drug delivery and unwanted modification of the effective properties of drug [4]. In comparison to aforementioned, artificial polymers may modify effective properties and chemical composition of drugs in drug delivery systems besides having other issues like cost, very poor biodegradability, etc [5].

These natural biopolymers are being developed for various purposes like bio-sensors, packaging, medicinal applications and in biomedical engineering [6]. Major examples of highly investigated bio-polymers are chitosan, lignin, cellulose, starch, alginate, gums, plant rubber, collagen, wool, heparin, silk, pectin, etc. [7]. Chitosan is broadly considered best candidate due to its specific linear structure and positive charge from N-atoms for donation which gives it unique features like adhesion to mucous membrane, catalytic effect on assimilation and dispersal process, immunogenicity, capability to encapsulate both hydrophilic and hydrophobic molecules and good clearance rate [8,9]. As Chitosan is a

natural polymer with living tissue, being easily degradable by nature, it cannot cause any allergic reaction or other side effect in the body during the drug release steps [10]. Chitosan is approved by US EPA for use in agriculture to enhance productivity. It is also a safe recognized food additive by Korean FDA since 1983 providing it potential employment in medicinal, dental, food, grooming fields [11,12]. Sodium Alginate is natural polysaccharide having many anionic or cationic groups in the structure.It is formed of mono or di-saccharides joined together by glycosidic bonds.It is an ideal carrier having features like biodegaradbility, non-toxicity, biocompatibility and cheap. It is widely used in drug-delivery applications [8, 13].Thymol(C10H14O), one of the major mono-terpenoids, GRAS notated by FDA, derived mainly from oregano and thyme (which is a major component detected in Thymus Vulgaris plant) has been proven to be having good inhibitory effect on fungi [14], microbes [15], cancerous cells [16], viruses [17], tumors [18], diabetes [18], inflammation [19], oxidation [20], controlling spasms [21] either alone or in association with other compounds [18]. It has very good dissolution ability in alcohol as well as such other organic solvents like acetone, ether etc. This work is aimed at determining the anti-inflammatory effect of thymol loaded sodium alginate-chitosan copolymeric nanoformulation as compared to pure thymol.We assessed that thymol loaded copolymeric nanoformulation had better anti-inflammatory effect than pure thymol.

Material and Method

Materials

Chitosan and Sodium-alginate were acquired from Hi-Media Private limited Mumbai (India); Thymol was obtained from Sigma-Aldrich. Lab-standard chemicals were employed throughout experiment. De-ionized water was used for buffers as well as solutions.

Preparation and Optimization of nanoformulation

To make copolymeric nanoformulation ionic gelation method was employed where the chitosan solution(0.1g, 0.01g, 0.06g) was readied by dispersing the known amount in 1%(v/v)strong acetic acid by constant stirring followed by dropwise addition of sodium alginate solution(0.1g, 0.01g,0.06g)prepared in purified distilled water. To which thymol solution of concentration of 4 mg/ ml in ethanol was introduced drop by drop with micropipette under continuous stirring. The mixture so made was kept for overnight stirring on magnetic stirrer at 600rpm at room temperature. Particle dimensions were studied by using Particle size analyzer (PSA) and percentage of entrapment (EE) by using UV-visible spectrophotometer. Further design-expert software (version 8.0.4, Stat Ease Inc.) was employed to determine the optimized value of concentration of polymers. Statistical analysis was conducted for evaluation of significance.Solution with smallest dimensions and highest EE was chosen as optimized nanoformulation for further characterization and assessment after lyophilization on Labconco, Freezone 6-plus for 24 hours.

Characterization

Encapsulation-Efficiency (EE)

After centrifuging the copolymeric nanoformulation for a time period of 30 minutes at rpm of 10,000 at a temp of 4°C on Remi Centrifuge C-30 Plus, analysis of supernatant using UV-visible spectrophotometer at 274 nm (Shimadzu Corporation, UV-2450) was conducted to determine free thymol. Encapsulation-efficiency of nanoformulation was evaluated using formula explained below.

$$\% \text{ encapsulation} = (Tt - Tf) / Tt \times 100$$

In above equation, Tt is total thymol, Tf is free thymol.

Size and stability

Zetasizer Nano ZS-90 (Malvern Instrument) was utilized to analyze size using dynamic light scattering technique and Zeta potential bylaser light scattering technique for determining stability formulation.

Invitro assessment ofAnti-inflammation

The inflammation reducing effect of various concentrations(500,250,125,62.5µg/ml)of thymol loaded copolymeric nanoformulation was assessed by HRBC stabilization method [22] and is also described in our previous study[23].T-test was further employed to statistically analyze the nanoformulation.

Result and discussion:

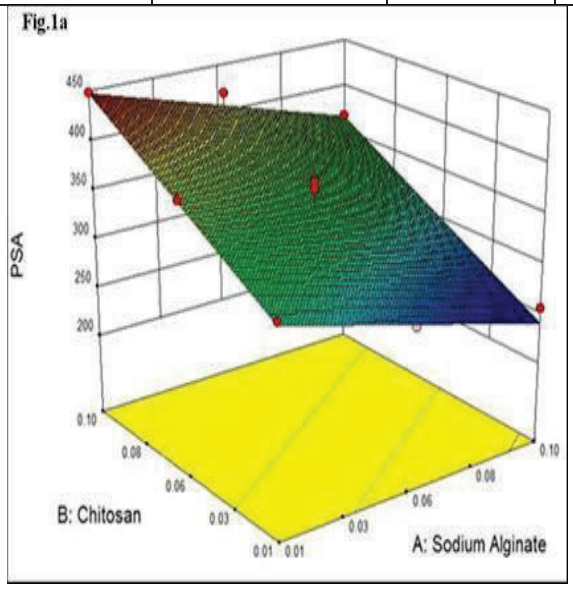
Optimization of nanoformulation

Design Expert software was used to obtain optimized concentration of polymers with desired responses for PS and EE (Table.1).Row 14 shows optimized batch having smallest PS and highest EE, which was used for further in vitro assessment. Output files from the software were used to build 3D surface plot for PS (Fig.1a) and EE (Fig.1b)which revealed, although concentration of both polymers affectedPS and EE butthe effect of concentration of chitosan was more pronounced than Sodium Alginate on both PS and EE.

Table.1 Experimental-trials for optimization of thymol loaded sodium alginate and chitosan copolymeric nanoformulation.

Run No.	Conc. of Sodium Alginate (g)(**A**)	Conc. ofChitosan (g) (**B**)	PS (nm) (**C**)	E.E. (%) (**D**)
1	0.1	0.01	256	91.5
2	0.01	.1	448	95.23
3	0.01	.01	324.8	86.23
4	0.06	.06	301.9	97.2
5	0.1	.06	294.2	95.9
6	0.01	.06	386	90.2
7	0.06	.06	342.2	96.8
8	0.1	0.1	368	98.8
9	0.06	0.06	321.4	98.2
10	0.06	0.06	363.1	96
11	0.06	0.01	278.3	88.89
12	0.06	0.1	420	99.2
13	0.06	0.06	371.2	97
14	0.1	0.05	209	89

Fig.1a



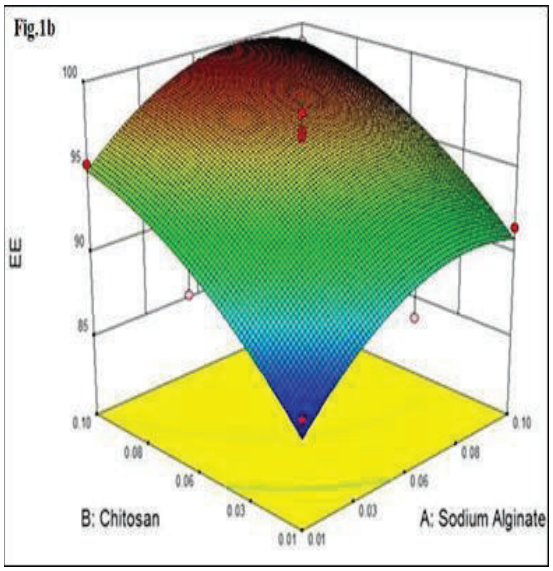
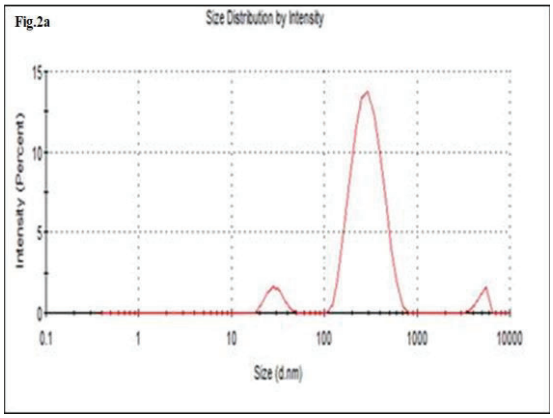


Fig.1a &1b: depicts 3D response surface plot for PS(size) and EE(encapsulation).

Size and Stability



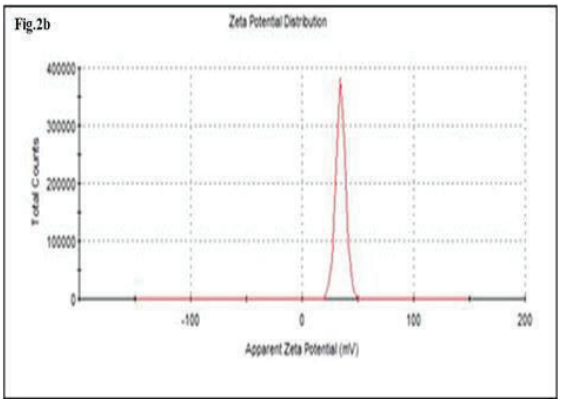


Fig.2a &2b: depicts the graph for average sizeand zeta potentialof optimized thymol loaded copolymeric nanoformulation.

The Z-average diameter of 209nm of an optimized thymol loaded copolymeric nanoformulation was obtained by dynamic light scattering(Fig.2a). Further stability of the optimized nanoformulation was tested by recording its zeta potential which was 34.5mV (Fig.2b).

Anti-inflammatory effect

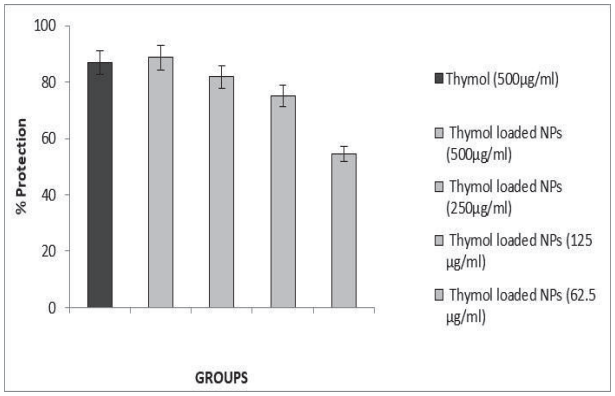


Fig.3: depicts the in-vitro anti-inflammatory effect of thymol and thymol loaded nanoformulation of different concentrations.

Anti-inflammatory effect of 500µg/ml pure Thymol was assessed as 86.8% while those of different concentrationsof Thymol-loaded NPs were determined as follows: 500µg/ml-88.7%, 250µg/ml-81.7%, 125µg/ml-74.9% and 62.5µg/ml-54.5%.These results prove that encapsulated thymol expresses greater inflammation inhibition characteristics than pure thymol, which are in line with various reported studies that NPs of Thymol have greater anti-inflammation potential than the pure thymol [23]. The significance of above four concentrations of NP was assessed from *p*-value obtained by *t*-test as shown in **Fig.3**. Thymol NPs at 500µg/ml concentration were found to have highest significance.

Conclusion

Optimized thymol-loaded nanoformulation was formed by Ionic gelation method using Sodium Alginate-Chitosan copolymer with highest Encapsulation efficiency of 89% at a ratio of as 2:1. Further characterization of optimized nanoformulation depicted size of 209nm and zeta-potential of 34.5mV.Maximum anti-inflammatory effect was found at 500µg concentration of thymol-loaded coplymeric nanoparticles*in-vitro*suggesting scope for studying *in vivo*as novel natural anti-inflammatory compound.

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